

ROLE OF RHIZOSPHERE MICROORGANISMS IN THE UPTAKE OF
NITROGEN BY PLANTS¹

by

J. O. LEGG AND F. E. ALLISON²

It has been recognized for many years that the region of soil under the immediate influence of plant roots is a site of intense biological activity. This relatively indefinite region generally is designated as the rhizosphere. The interrelations between microorganisms and plant roots in the rhizosphere has recently been reviewed by Starkey (9). Among these interrelations, the effects of microorganisms on the availability of nutrients in the rhizosphere have been studied to a certain extent, but few results of a quantitative nature have been produced. With respect to N availability, it is generally recognized that at least two important factors are involved, namely, (a) biological interchange (immobilization and mineralization) which takes place both in the presence and in the absence of plants, and (b) the rhizosphere effect which is superimposed on ordinary biological interchange in the presence of plants. The purpose of this paper is to present data which shed light primarily on (b).

MATERIALS AND METHODS

The initial results in this work were obtained in a greenhouse study of ammonium fixation in soils, and the experimental procedures are published elsewhere (6). In general, Sudangrass was grown in pots containing 3 kg. of soil at 6 N levels, tagged with N¹⁵. Plant uptake of total and N¹⁵-tagged N was determined by Kjeldahl and mass spectrometer analyses, and soil N uptake was obtained by difference.

Subsequent experiments were carried out in a growth chamber where light and temperature were carefully controlled. Oats were grown in plastic pint containers filled with quartz sand containing varying levels of ordinary N and sufficient amounts of other essential elements. After the plants had grown for relatively short periods, usually 17 days, a constant amount of N¹⁵-tagged N was added to all containers. After given uptake periods the plants, including roots, were harvested and analyzed for total and isotopic N.

RESULTS

The greenhouse experiments were designed primarily for the study of N uptake by plants from an ammonium source. Since the total N in the plants was easily separated into the fractions arising from each of the two sources, soil and fertilizer, both values were obtained and are presented in table 1. The values include N from both the roots and the tops of the plants. The soils in this experiment were obtained from the B horizons of the Myersville and Miami series, and consequently were low in soil N content. Thus, the amount of soil N taken up by the plants is relatively low; however, the increase in soil N uptake as the fertilizer N was increased is relatively large. The Miami soil supplied less soil N to the plants than the Myersville

¹ Contribution from the Soil and Water Conservation Research Division, Agricultural Research Service, USDA, Beltsville, Md.

² Soil Scientists.

III.12

soil, but the growth period was 8 weeks for the Miami soil as compared to 12 weeks for the Myersville. Both soils responded similarly in that the difference in the uptake of the soil N at the low and high fertilizer N rates was approximately three-fold in both cases. The apparent relationship which existed between the uptake of soil N and the N percentage of the plants is illustrated in figure 1 for the Myersville soil. The correlation coefficient is highly significant.

TABLE 1. Uptake of soil and fertilizer N from two soils by Sudangrass as influenced by the rate of fertilization with N^{15} -tagged $(NH_4)_2SO_4$.

N rate (mg./pot)*	N uptake by Sudangrass (mg.)**			
	Myersville soil		Miami soil	
	Fertilizer N	Soil N	Fertilizer N	Soil N
0	0	22.6	0	9.5
37.5	14.2	34.0	15.0	16.2
75	35.8	41.6	38.6	19.4
150	88.4	53.4	86.4	24.4
300	201.2	61.4	185.7	30.1
600	457.3	74.5	391.0	34.8

* Highest rate equivalent to 400 lbs./acre.

** Average of 3 replications.

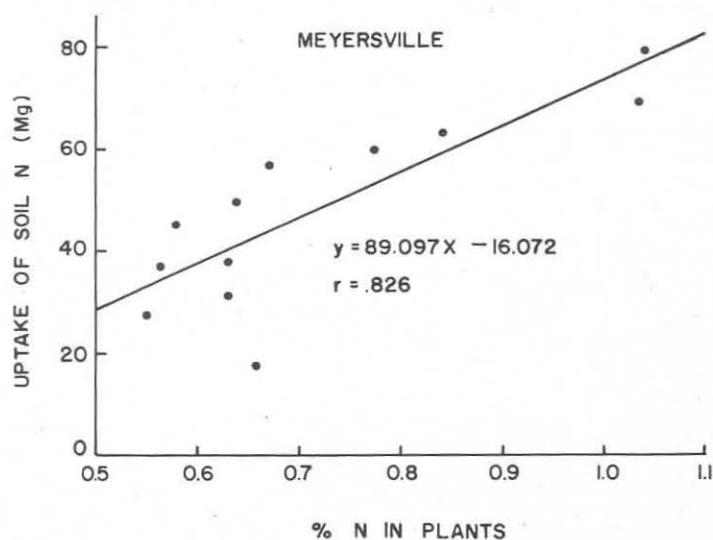


Figure 1. The relationship between the uptake of soil nitrogen by Sudangrass and the nitrogen percentage of the plants fertilized with varying amounts of $(N^{15}H_4)_2SO_4$.

In order to investigate possible reasons for the observed differences in soil N uptake, a series of sand-culture experiments was undertaken. This obviously eliminated mineralization-immobilization reactions of the soil organic N as factors. Furthermore, since the plants were first grown at

varying N levels, using an untagged source, the uptake of subsequent, single-level, additions of N^{15} -tagged N was of primary interest.

Table 2 shows the results of the first sand-culture experiment. It is apparent that the initial N level of the plant influenced greatly the subsequent uptake of N^{15} additions. Differences in the size of plants could be expected

TABLE 2. Uptake of tagged nitrogen by oats grown at varying nitrogen levels for 17 days, then for 3 days with an additional 7 mg. nitrogen as $(N^{15}H_4)_2SO_4$.

N. level mg./pot	Plant wt. (g.)	N content		% recovery of tagged N
		mg.	%	
Roots				
0	1.041	6.37	0.61	16.88
5	1.343	9.02	.67	19.14
10	1.291	9.32	.72	24.24
20	1.765	12.10	.69	24.37
35	1.469	14.24	.97	28.27
50	1.466	16.23	1.11	30.00
Tops + roots				
0	1.658	18.98	1.14	38.46
5	2.347	26.52	1.13	40.87
10	2.518	29.98	1.19	49.09
20	3.252	39.38	1.21	50.24
35	3.232	53.67	1.66	63.74
50	3.169	65.14	2.06	70.65

to influence N uptake, especially in short-term absorption periods; however, an examination of the data shows that the 20 mg. level produced the largest plants, but the uptake of N^{15} at this level was substantially lower than that at the 50 mg. level.

A similar experiment was carried out in which the N^{15} uptake period was varied from 12 hours up to 8 days. The results obtained with plants grown initially with additions of 0, 20, or 50 mg. of ordinary N for 17 days, followed by 10 mg. additions of N^{15} -tagged N to all pots, are shown, in figure 2. Again a direct relationship appears to exist between the N level of the plant and the subsequent uptake of tagged N. The range of differences in N^{15} uptake became somewhat less at 8 days than at shorter uptake periods, but the general uptake pattern remained the same.

CONCLUSIONS

Although N^{15} has been used rather widely in agronomic research in recent years, little direct evidence has accumulated concerning the effect of fertilizer rates on soil N utilization by plants. Apparently Walker et al (10) in 1956 were the first workers to point out that the uptake of soil N by plants may be increased by increasing fertilizer N rates. Little significance was attached to this finding, however, other than to attribute it to the normal turnover between the fertilizer N and the soil organic N. In earlier tracer experiments by others (1, 2, 3, 7) where sufficient data have been given, it is possible to calculate the effect of N additions on soil N uptake. Such indirect evidence shows that it is a common occurrence for soil N uptake to increase with additions of N^{15} -tagged fertilizers. The data shown in table 1 therefore substantiate and reemphasize the work of others which, for the most part, has remained rather obscure in the past.

III.12

The work of Goring and Clark (4), particularly, suggested that the results presented in table 1 could be explained on the basis of immobilization reactions in the rhizosphere. In nontracer experiments they found that *net mineralization* of soil N is less in cropped than in uncropped soils, the N in the plants being taken into account. They also showed a direct correlation between the N content of plant roots and the total uptake of N. A similar relationship exists between the N content of plants and the uptake of soil N, as shown in figure 1.

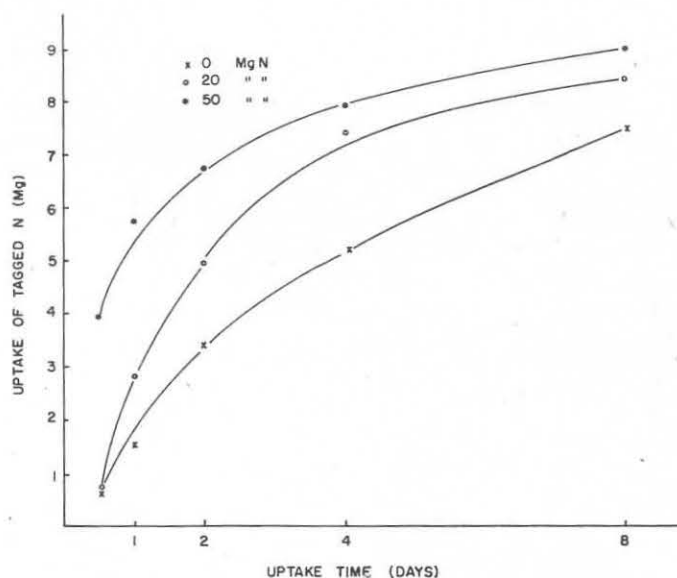


Figure 2. The effect of N content of oat plants on the uptake of tagged-N added after 17 days.

Since soil organic N was eliminated in the sand cultures, it appears that the differences in N^{15} uptake at the different plant N levels (table 2, fig. 2) can be accounted for primarily through immobilization of the N^{15} additions by rhizosphere microorganisms. The percent N in the roots of plants grown at the different N levels indicates the wide range in C/N ratios of roots grown under such conditions. It is well known that the microbial population is much higher in the rhizosphere than in the rest of the soil, presumably because of the energy material derived from roots. That plant roots excrete a number of organic compounds including amino acids, sugars, phosphatides, alkaloids, tannins, and vitamins is well established (9) and, in addition, supply a source of energy material in the form of sloughed-off root hairs, epidermal cells, and dead roots. Quantitative estimates of the various root excretions are lacking, but the large quantity of roots added to the soil by growing plants is demonstrated by the work of Pinck, et al (8). They found that during a 3—4 year period, with several crops grown in the greenhouse, the addition of moderate amounts of N alone gave a 24 per cent increase in soil C due to root residues. Plants grown at low N levels thus furnish a large amount of material with a high C/N ratio to the growth medium which, through the action of microorganisms, immobilizes mineral N needed by the plants. That there is a gradual turnover of the immobilized

N is indicated by the slow but continued uptake of N^{15} near the end of the 8-day uptake period (fig. 2). With a more mature plant system in soil, as generally encountered in plant tests, it could be expected that N turnover would be slower because of less readily decomposable materials.

The influence of rhizosphere microorganisms on the uptake of N by plants readily explains some of the conflicting reports, reviewed briefly elsewhere (4), concerning the effect of growing plants on net mineralization of organic N in soils. In a soil which supplies the growing plant with sufficient N to maintain a favorable root C/N ratio, the rhizosphere micro-organisms require little or no additional N for the decomposition of root excretions and residues. On the other hand, as the capacity of the soil supply N to the plant decreases, the C/N ratio of the plant increases, and the demand of rhizosphere microorganisms for additional mineral N increases correspondingly. In the latter case the growing plant exerts a depressing effect on net mineralization of soil organic N as compared to net mineralization in fallow soil.

The rhizosphere effect also helps to explain the buildup of organic matter in grassland soils, and the characteristically low level of mineral N in such soils even shortly after additions of N in periods when little growth of grass takes place. This phenomenon has been well described by Harmsen and van Schreven (5).

From a practical standpoint, knowledge of the magnitude of the rhizosphere effect, as indicated by tracer experiments, should be of value in making better predictions of the probable response of soils to N fertilizers from nitrification tests. Such information should also be useful in devising the best means of utilizing both fertilizer and native soil N in crop production.

REFERENCES

1. BARTHOLOMEW, W. V., and A. E. HILTBOLD, Recovery of fertilizer nitrogen by oats in the greenhouse. *Soil Sci.* **73**: 193—201. 1952.
2. BARTHOLOMEW, W. V., L. B. NELSON, and C. H. WERKMAN, The use of nitrogen isotope N^{15} in field studies with oats. *Agron. Jour.* **42**: 100—103. 1950.
3. BROADBENT, F. E., and A. G. NORMAN, Some factors affecting the availability of the organic nitrogen in soil — a preliminary report. *Soil Sci. Soc. Amer. Proc.* (1946) **11**: 264—267. 1947.
4. GORING, C. A. I., and F. E. CLARK, Influence of crop growth on mineralization of nitrogen in the soil. *Soil Sci. Soc. Amer. Proc.* (1948) **13**: 261—266. 1949.
5. HARMSSEN, G. W., and D. A. VAN SCHREVEN, Mineralization of organic nitrogen in soil. *Advances in Agron.* **7**: 299—398. 1955.
6. LEGG, J. O., and F. E. ALLISON, Recovery of N^{15} -tagged nitrogen from ammonium-fixing soils. *Soil Sci. Soc. Amer. Proc.* **23**: 131—134. 1959.
7. MACVICAR, R., W. L. GARMAN, and R. WALL, Studies on nitrogen fertilizer utilization using N^{15} . *Soil Sci. Soc. Amer. Proc.* (1950) **15**: 265—268. 1951.
8. PINCK, L. A., F. E. ALLISON, and V. L. GADDY, Utilization of nitrogen in cropping systems with and without green manure in the greenhouse. *Soil Sci.* **66**: 39—52. 1948.
9. STARKEY, R. L., Interrelations between microorganisms and plant roots in the rhizosphere. *Bacteriol. Rev.* **22**: 154—172. 1958.
10. WALKER, T. W., A. F. R. ADAMS, and H. D. ORCHISTON, Fate of labeled nitrate and ammonium nitrogen when applied to grass and clover grown separately and together. *Soil Sci.* **81**: 339—351. 1956.

SUMMARY

In greenhouse tests with N^{15} -tagged fertilizers, differences in the uptake of soil N by Sudangrass at low and high fertilizer N rates were approximately

III.12

three-fold on two soils. In further tests, carried out in sand culture, plants were first grown at different N levels (untagged), and then the uptake of N^{15} from subsequent additions of N^{15} -tagged N was determined. The results showed that the uptake of tagged N varied directly with the initial plant N level, thus corresponding with the N uptake data from soils. The observed differences in uptake of N by plants is attributed primarily to differences in the immobilization of mineral N by rhizosphere microorganisms in the utilization of energy material derived from roots of varying C/N ratios.

RÉSUMÉ

Au cours d'essais en serre avec des engrais marqués au N^{15} , il fut trouvé que pour deux sols l'assimilation d'azote du sol par Sudan grass fut environ trois fois plus élevée en présence de fortes doses d'engrais qu'en présence de faibles doses. Dans une expérience suivante, les plantes furent d'abord établies dans des cultures de sable à des niveaux croissants d'azote (non marqué). Ensuite l'adsorption de N^{15} à partir d'engrais marqué fut déterminé. Les résultats montrent que l'assimilation d'azote marqué était en raison directe du taux d'azote initial de la plante, correspondant ainsi à l'assimilation d'azote à parir du sol. Les différences entre quantités adsorbées par les plantes doivent être attribuées en premier lieu à des différences dans l'immobilisation d'azote minéral par les organismes de la rhizosphère qui utilisent des déchets radiculaires à rapports C/N variés comme matériel énergétique.

ZUSAMMENFASSUNG

Bei im Gewächshaus angestellten Versuchen mit durch N^{15} markierten Düngermitteln waren die Unterschiede in der Aufnahme des Boden-N durch Sudangras auf zwei Böden bei niedrigen und hohen Gaben an Dünger-N annähernd dreifach. In weiteren Versuchen in Sandkultur ausgeführt, wurden Pflanzen erst bei verschiedenen N-Gehalten (jedoch noch nicht markiert) gezüchtet; und dann wurde von folgenden Zufügungen von mit N^{15} markiertem N die Aufnahme von N^{15} bestimmt. Die Resultate zeigten, dass die Aufnahme von markiertem N direkt mit der anfänglichen Menge pflanzenaufnehmbaren Stickstoffs variierte, somit korrespondierte mit den Aufnahmedaten des N aus den Böden. Die beobachteten Unterschiede in der N-Aufnahme durch Pflanzen sind demnach an erster Stelle Differenzen in der Immobilisation von mineralischem N durch Mikroorganismen der Rhizosphäre, und zwar in der Ausnutzung von Energiematerial verschiedener C/N-Verhältnisse, den Wurzeln entzogen, zuzuschreiben.

DISCUSSION

H. KATZNELSON: Do you actually have evidence of a change in the C/N ratio of roots as a result of different levels of nitrogen fertilization of soil?

J. O. LEGG: A two-fold change in the present N in the roots in the sand-culture experiment (Table 2) indicates the change in C/N ratio as the N level is varied.

H. KATZNELSON: Is there any evidence that the rhizosphere microflora does indeed immobilize some of the nitrogen added? If so does the use of N^{15} indicate how much?

J. O. LEGG: Yes. In sand-culture experiments, where mineralization-immobilization reactions of the soil organic matter were eliminated, the uptake of N^{15} -tagged N was dependent on the initial N level of the plant. In this case the difference in uptake of N^{15} by plants at low and high N levels indicates the amount of immobilization. In soils, where N^{15} -tagged fertilizers are added initially at different rates, the differences in soil uptake at low and high fertilizer rates indicate the amount of soil N immobilized.